

Dibromoethane Effects on the Induction of
Gamma-Glutamyl-Transpeptidase
Positive Foci in Rat Liver

Ohio State Univ., Columbus

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1982



U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA (Please read instructions on the reverse before complet)		
1. REPORT NO. EPA-600/J-82-272	2. JOURNAL ARTICLE	3. PB83-157552
4. TITLE AND SUBTITLE Journal Article: Dibromoethane Effect on the Induction of Gamma Glutamyltranspeptidase Positive Foci in Rat Liver		5. REPORT DATE 1982
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) M. M. Milks, S. R. Wilt, I. Ali, M. A. Pereira and D. Couri		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Ohio State University College of Medicine Columbus, Ohio 43210		10. PROGRAM ELEMENT NO. CCSLIA
		11. CONTRACT/GRANT NO. CR-807378
12. SPONSORING AGENCY NAME AND ADDRESS U. S. Environmental Protection Agency Health Effects Research Laboratory Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES Archives of Toxicology 51:27-35 (1982) (Project Officer: Michael A. Pereira)		
16. ABSTRACT The initiating and promoting activities of 1,2-dibromoethane in rat liver were investigated using the enzyme-altered foci bioassay. The incidence of γ-glutamyl-transpeptidase (GGT)-positive foci was used as an early histochemical marker for hepatocarcinogenesis. To determine the initiating activity of 1,2-dibromoethane, the halogenated hydrocarbon was administered orally in corn oil as single or multiple doses (60 or 120 mg/kg) either before or after partial hepatectomy. The animals were then given a promoting regimen of 500 ppm phenobarbital in their drinking water. No increase in the incidence of GGT-positive foci was observed in any of the 1,2-dibromoethane initiation groups. The tumor promoting activity of 1,2-dibromoethane was determined in partially hepatectomized rats which were initiated with N-nitrosodiethylamine (30 mg/kg; po), and one week later were administered 1,2-dibromoethane (10 or 30 mg/kg) orally in corn oil five times weekly for 8 weeks. Control groups receiving sham hepatectomy or no initiator were also treated with the halogenated hydrocarbon five times weekly. Only in those animals which received partial hepatectomy, N-nitrosodiethylamine initiation, and 1,2-dibromoethane was the incidence of GGT-positive foci significantly increased. These results do not support significant initiator activity of 1,2-dibromoethane in rat liver, but do indicate that 1,2-dibromoethane possesses promoter activity which may contribute to its carcinogenic activity.		
17.		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA 22161		
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 10
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

JOURNAL ARTICLE:

**Dibromoethane Effects on the Induction
of γ -Glutamyl-Transpeptidase Positive Foci in Rat Liver**

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Abstract. The initiating and promoting activities of 1,2-dibromoethane in rat liver were investigated using the enzyme-altered foci bioassay. The incidence of γ -glutamyl-transpeptidase (GGT)-positive foci was used as an early histochemical marker for hepatocarcinogenesis. To determine the initiating activity of 1,2-dibromoethane, the halogenated hydrocarbon was administered orally in corn oil as single or multiple doses (60 or 120 mg/kg) either before or after partial hepatectomy. The animals were then given a promoting regimen of 500 ppm phenobarbital in their drinking water. No increase in the incidence of GGT-positive foci was observed in any of the 1,2-dibromoethane initiation groups. The tumor promoting activity of 1,2-dibromoethane was determined in partially hepatectomized rats which were initiated with N-nitrosodiethylamine (30 mg/kg; po), and one week later were administered 1,2-dibromoethane (10 or 30 mg/kg) orally in corn oil five times weekly for 8 weeks. Control groups receiving sham hepatectomy or no initiator were also treated with the halogenated hydrocarbon five times weekly. Only in those animals which received partial hepatectomy, N-nitrosodiethylamine initiation, and 1,2-dibromoethane was the incidence of GGT-positive foci significantly increased. These results do not support significant initiator activity of 1,2-dibromoethane in rat liver, but do indicate that 1,2-dibromoethane possesses promoter activity which may contribute to its carcinogenic activity.

Key words: 1,2-dibromoethane – Phenobarbital – N-nitrosodiethylamine – γ -Glutamyl-Transpeptidase – Promotion

Introduction

The halogenated hydrocarbon 1,2-dibromoethane (ethylene dibromide) is a widely used compound which has pronounced biological effects. Although the

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principal uses of 1,2-dibromoethane are as a lead scavenger in gasoline and as a pesticide in soil and grain fumigants, this compound is also used in lesser amounts as an industrial solvent and as a chemical intermediate in the syntheses of dyes, pharmaceuticals, and other organic compounds (IARC 1977). The toxic effects of 1,2-dibromoethane in rats are known to include central nervous system depression, pulmonary irritation, hepatic and renal injury, and in hens, decreased egg laying (Rowe et al. 1952; Bondi et al. 1955; Nachtomi et al. 1968; Broda et al. 1976). Other important toxicities of 1,2-dibromoethane include mutagenicity (Brem et al. 1974; Fishbein 1976; Rannug 1980) and carcinogenicity (Olson et al. 1973; Van Duuren et al. 1979; Stinson et al. 1981). That dibromoethane covalently binds to DNA, RNA, and protein has been established (Nachtomi and Sarma 1977; Hill et al. 1978; Banerjee et al. 1979). However, due to its proliferative stimulus to rat hepatocytes (Nachtomi and Farber 1978), 1,2-dibromoethane could conceivably possess significant tumor promoter activity in addition to its apparent genotoxicity. For this reason we examined the initiating (genotoxic) and promoting (epigenetic) effects of 1,2-dibromoethane in the rat liver foci bioassay using γ -glutamyl-transpeptidase as a histochemical marker for the enzyme-altered foci (Scherer and Emmelot 1976; Solt et al. 1977; Tsuda et al. 1980; Ford and Pereira 1980; Couri et al. 1982; Pereira 1982).

Materials and Methods

Chemicals

1,2-Dibromoethane (99% pure) was purchased from Aldrich Chemical Company, Milwaukee, WI, USA; N-nitrosodiethylamine from Sigma Chemical Company, St. Louis, MO, USA; phenobarbital sodium from J. T. Baker Chemical Company, Phillipsburg, NJ, USA; ether for anesthesia U.S.P. from Mallinckrodt, Inc., St. Louis, MO, USA; gamma-glutamyl-4-methoxy-2-naphthylamide from Bachem Inc., Torrance, CA, USA; O.C.T. embedding compound from VWR Scientific Inc., Columbus, OH, USA; and hematoxylin from Fisher Scientific Company, Fair Lawn, NJ, USA.

Animals

Male Sprague-Dawley rats (140 ± 10 g, were obtained from Harlan, Indianapolis, IN, USA and were housed in American Association for Accreditation of Laboratory Animal Care (AAALAC) approved facilities under climate-controlled conditions of a 12 h light cycle (6 a.m.–6 p.m.), room temperature of $22-23^\circ\text{C}$, and relative humidity of 45%. All animals received Purina Lab Chow and tap water ad libitum.

Experimental Design

Initiation Protocol. The initiation protocol was intended to investigate the genotoxic effect of 1,2-dibromoethane in liver. Four consecutive daily doses of 60 mg/kg 1,2-dibromoethane were given orally in corn oil either before (Groups 1 and 2) or after (Groups 3 and 4) partial hepatectomy, or a single 120 mg/kg dose of 1,2-dibromoethane was administered orally to partially hepatectomized rats

(Groups 6 and 7). A positive control group (Group 8) was also included which received partial hepatectomy, followed 24 h later with a single oral dose of 30 mg/kg N-nitrosodiethylamine. Three days following administration of the initiator, the rats received 500 ppm phenobarbital in their drinking water for a total of 56 days. The animals were then sacrificed and the livers scored for GGT-positive foci.

Promoting Protocol. This protocol allowed examination of the promoting activity of the halogenated hydrocarbon. Following a partial hepatectomy, the rats received 30 mg/kg N-nitrosodiethylamine (Groups 9 and 10) or water vehicle (Groups 11 and 12). A sham operated group (Group 13) was also initiated with N-nitrosodiethylamine. Three days subsequent to initiation, the animals were started on the promoting regimen of either 10 or 30 mg/kg 1,2-dibromoethane given in corn oil by oral gavage five times weekly. Two additional control groups were included which received promoter vehicle (corn oil, Group 14; or tap water, Group 15). The rats were sacrificed after 8 weeks of dibromoethane treatment and the livers scored for GGT-positive foci.

Analytical Procedures

Animals were sacrificed and slices (approximately 2 mm) were obtained from the right lateral and the caudate lobes of the liver. The liver slices were embedded in O.C.T. compound and rapidly frozen by placing the tissue molds on dry ice. The frozen blocks were stored at -80°C until cryostatic sections were prepared within 1 week. Six-micron sections were mounted on glass microscope slides and stained for γ -glutamyl-transpeptidase (GGT) activity (Rutenburg et al. 1969). The slides were then counterstained with hematoxylin. GGT-positive foci of at least nine cells or more were scored. The area of the liver section examined was calculated by projecting the section image onto paper and tracing the outline with a planimeter.

Results

Initiation

The results of the initiation treatments are summarized in Fig. 1, Groups 1–8. Of the groups of rats in which 1,2-dibromoethane was tested as an initiator, (Groups 1–7), none showed an induction of GGT-positive foci above levels found in non-initiated rats treated with phenobarbital (approximately 0.7–1.1 foci/cm²; data not shown). The N-nitrosodiethylamine positive control treatment (Group 8) resulted in 4.7 foci/cm². These results suggest that 1,2-dibromoethane at doses up to 120 mg/kg was without significant initiating activity in rat liver.

Promotion

The promoting activity of 1,2-dibromoethane is demonstrated in Fig. 2, Groups 9–15. When 1,2-dibromoethane was administered five times weekly for 8 weeks following initiation with 30 mg/kg N-nitrosodiethylamine, (Groups 9 and 10), a significant increase in foci production resulted. Neither promoting dose (10 nor 30 mg/kg) of 1,2-dibromoethane produced foci above control levels in rats not initiated with N-nitrosodiethylamine (Groups 11 and 12). Likewise, the sham operation group (Group 13) which received N-nitrosodiethylamine and the 30

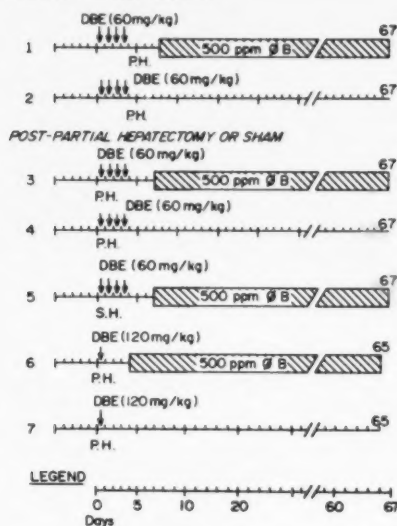
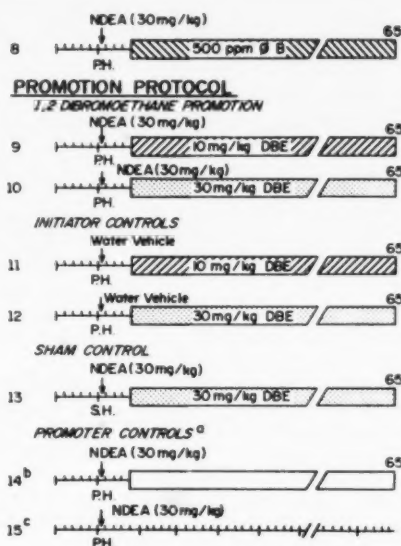
INITIATION PROTOCOL**PRE-PARTIAL HEPATECTOMY****POSITIVE CONTROL**

Fig. 1. Summary of experimental protocol. Groups of male Sprague-Dawley rats (130–150 g) were treated according to the protocols outlined above. After 8 weeks of promotion, the rats were sacrificed and fresh frozen sections of liver were stained and examined for focal enzyme activity of γ -glutamyl-transpeptidase (GGT). (■) 500 ppm ϕ B-500 ppm Phenobarbital in drinking water; (▨) 10 mg/kg DBE-10 mg/kg 1,2-Dibromoethane administered in corn oil by gavage five times weekly; (□) 30 mg/kg DBE-30 mg/kg 1,2-Dibromoethane administered in corn oil by gavage five times weekly; (□) Corn oil administered by gavage five times weekly. NDEA – N-Nitrosodiethylamine administered in distilled water by gavage; P.H.-2/3 Partial Hepatectomy; S.H.-Sham Hepatectomy ^aPromoter control groups from an earlier experiment; ^bCorn oil vehicle promoter control; corn oil was administered by gavage five times weekly; ^cTap water vehicle promoter control; drinking water was normal tap water

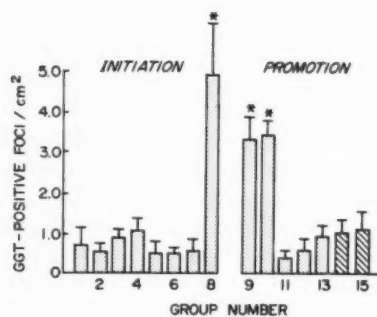


Fig. 2. Initiating and promoting effects of 1,2-dibromoethane on enzyme-altered foci formation. Groups of male Sprague-Dawley rats (130–150 g) received the treatments as described earlier in the methods section and outlined in Figure 1. Histochemical staining of cryostatic sections of liver revealed areas of GGT activity indicating enzyme-altered foci. Results are expressed as the mean number of foci ($\pm$ SEM) per square centimeter of tissue examined.

*Significantly greater than vehicle promoter controls (groups 14 and 15); student's *t*-test, $p < 0.02$

mg/kg 1,2-dibromoethane promotion regimen did not show any increase in GGT-positive foci. Corn oil and water vehicle treated groups given N-nitrosodiethylamine and partial hepatectomy resulted in only low numbers of foci (Groups 14 and 15). These results demonstrate that 1,2-dibromoethane possesses the ability to enhance the incidence of N-nitrosodiethylamine-initiated GGT-positive foci.

Discussion

The phenomenon of tumor promotion, first described in both rabbit and mouse skin by Rous and Kidd (1941), Berenblum (1941), and Friedewald and Rous (1944), has been applied to various tissues and organ systems (Colburn 1980). Peraino et al. (1971) have shown that hepatocarcinogenesis can likewise be divided into at least two discrete stages: (1) *initiation* — accomplished with the liver carcinogen 2-acetylaminofluorene and (2) *promotion* — accomplished by phenobarbital. As a liver tumor promoter, phenobarbital has received considerable investigation with respect to its mechanism, kinetics, and interactions in the process of hepatocarcinogenesis (Rossi et al. 1977; Peraino et al. 1978; Watanabe and Williams 1978; Peraino et al. 1980; Shinozuka and Lombardi 1980; Schulte-Hermann et al. 1981). Other drugs and environmental agents have also been implicated as potential promoters of liver cancer, among which are oral contraceptive steroids (Yager and Yager 1980), methapyrilene (Couri et al. 1982), polychlorinated biphenyls (Preston et al. 1981; Pereira et al. 1982a), hexachlorocyclohexane (Schulte-Hermann et al. 1981; Pereira et al. 1982b), hexachlorobenzene (Pereira et al. 1982b) and 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (Pitot et al. 1980). Other measures such as repeated partial hepatectomy (Pound and McGuire 1978) and choline-devoid diet (Shinozuka et al. 1979) have also been found to stimulate the process of hepatocarcinogenesis.

Recently, various histochemical techniques have been developed and applied toward identifying early putative preneoplastic foci in liver (Scherer and Emmelot 1975; Solt et al. 1977; Pitot 1977; Pitot et al. 1978; Hirota and Williams 1979; Ogawa et al. 1980; Solt et al. 1980; Bannasch et al. 1980; Emmelot and Scherer 1980; Pitot and Sirica, 1980; Farber 1980; Williams 1980). One such histochemical technique involves the focal demonstration of the enzyme γ -glutamyl-transpeptidase. The occurrence of GGT-positive foci is used to estimate the probable carcinogenic activity of various chemicals (Boelsterli 1979; Williams et al. 1980; Ford and Pereira 1980; Tsuda et al. 1980; Pereira 1982).

Despite the documented genotoxicity of 1,2-dibromoethane (Fishbein 1976; Nachtomi and Sarma 1977; Hill et al. 1978; Banerjee et al. 1979; Rannug 1980), the halogenated hydrocarbon was without initiating activity in the hepatic enzyme-altered foci bioassay. It has been reported that partial hepatectomy greatly enhances initiation of chemical carcinogenesis (Scherer and Emmelot 1975; Cayama et al. 1978; Hirakawa et al. 1979; Ishikawa et al. 1980; Columbano et al. 1981). When 1,2-dibromoethane was administered before or after partial

hepatectomy, there was still no initiation of enzyme-altered foci. Hill et al. (1978) have reported that the irreversible binding of radioactivity from (^{14}C)-1,2-dibromoethane to DNA was four times greater in liver than in the target organ (stomach). The total DNA binding in these two tissues did not correlate with the observed tumorigenic response in the chronic carcinogenesis bioassay. The difference in the rate of cell turnover in the two tissues may explain the increased carcinogenic effect seen in stomach as compared to liver. However, in the experiments reported here, the lack of response in liver cannot be explained by a low turnover rate of the cells, since partial hepatectomy dramatically increases hepatocyte turnover. The reason for this lack of initiator activity of 1,2-dibromoethane in liver is not yet clear, but may be related to rapid and efficient liver DNA repair, or to the specific nature of the adducts formed when 1,2-dibromoethane binds to DNA.

Tumor promotion has been associated with hyperplasia (Boutwell 1974; Schulte-Hermann 1974; Schulte-Hermann et al. 1981), and 1,2-dibromoethane has been demonstrated to act as a mitogen in rat liver (Nachtomi and Farber 1978). In N-nitrosodiethylamine treated rats, 1,2-dibromoethane was found to enhance the incidence of GGT-positive foci (Fig. 2). These results suggest that 1,2-dibromoethane is a tumor promoter in rat liver and that the promoter activity of 1,2-dibromoethane may be related to its mitogenic effect.

In summary, 1,2-dibromoethane was found to possess promoter activity in the rat hepatic enzyme-altered foci bioassay, but was without significant initiator activity. These results suggest that 1,2-dibromoethane possesses epigenetic (promoter) activity which may contribute to its carcinogenic effect.

Acknowledgements. This research was supported in part by cooperative agreement CR 807378 with the U.S. Environmental Protection Agency, Cincinnati, Ohio. The authors wish to thank John A. Young and Emma Garcia for their fine technical assistance. Also appreciated are the efforts of Beth Keller and Gretchen Lowry in typing this manuscript.

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Received March 16, 1982